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(54) [Title of the invention] The method of measuring IgE in blood

(57) [Summary]
[Constitute] A method of evaluation of the presence or the quantity of IgE from blood or blood component; after blood or blood component were diluted with a suitable solution, and then these were reacted with a soluble α chain of high affinity IgE receptor (FcεRI), and the presence or the quantity of the complex of IgE and a soluble α chain of Fc RI are measured, and finally the presence of IgE or the quantity of IgE is evaluated.

[Effect] Active IgE in blood and blood component can be detected or measured with ease.

[Range of patent application]

[Claim clause 1]A method of evaluation of the presence or the quantity of IgE from blood or blood component; after blood or blood component were diluted with a suitable solution, and then these were reacted with a soluble a chain of high affinity IgE receptor (FcεRI), and the presence or the quantity of the complex of IgE and a soluble α chain of FcεRI are measured, and finally the presence of IgE or the quantity of IgE is evaluated.

[Claim clause 2] Blood and Blood component as described previously include whole blood, serum and plasma.

[Claim clause 3] Anti-IgE antibody is secured onto a solid phase, and this solid phase is put into contact with the sample or the diluted sample, and if this sample contains IgE, this would be binded by the anti-IgE antibody which would be fastened to the solid phase. Next, this solid phase is put into contact with soluble α chain of FcεRI, leading to the formation of a bond between this soluble α chain of FcεRI and the solid phase. The presence or quantity of the soluble α chain of FcεRI determines the presence or quantity of the IgE in the sample.

[Claim clause 4] Soluble a chain of FcεRI is secured onto a solid phase, and this solid phase is put into contact with the sample or the diluted sample, and if this sample contains IgE, this would form a bond with the soluble α chain of FcεRI which would be fastened to the solid phase. Next, this solid phase is put into contact with anti-IgE antibody, leading to the formation of a bond between this anti- IgE antibody and the solid phase. The presence or quantity of the anti-IgE antibody determines the presence or quantity of the IgE in the sample.

[Detailed description of the invention]
[0001]
[Use on industrial field] The present invention is the method of measurement of IgE in human blood using FcεRI

[0002]
[Usual technique] Recently, an increase in allergy diseases is being reported in all over the world. In our country, these diseases were reported to be few percent in the1960's. However, currently it has increased to 10~20%, and is assumed to reach 30% by the end of this century. Allergic reactions refer to the damage of self tissues caused by the immune reaction, and are classified into four groups(type I, II, III and IV). In the present application, most of the allergic disease implies type I allergy.

[0003] In the type I allergy, when allergy antigens (allergens) invade the body, specific IgE antibody against the antigens bind to it. Prior to any antigen-antibody reaction, these IgE antibodies are attached via the Fc portion to the high affinity IgE receptor (FcεRI) on mast cell or basophil, and then following the binding of the allergen to IgE antibody, and aggregation of the receptor and degranulates were occurred, and finally normal allergic

reactions are induced by releasing of chemical mediators such as histamine and leucotriene.

[0004] The blood serum concentration of IgE antibody which causes type I in normal human is below 150~300u/ml, however, a patient afflicted by an allergic disease of atopy may contain 102~104 times this. In general, an increase in total IgE in type I allergy patient are well known. Measuring the total IgE or antigen-specific IgE plays an important role in diagnosis of allergic diseases.

[0005] Currently there are methods measuring IgE using IgE specific antibodies (monoclonal or polyclonal antibody). For example, it is possible to measure IgE levels through the use of RIA or ELISA. However, these method are not able to discriminate between functional and non-functional IgE found in the blood. In fact, as long as antigen-antibody reactions are being used, IgE's that lack the ability to be bound by the receptor on mast cell or basophil are detected. Furthermore, degenerated or denatured products are also detected. In other words, the IgE which is not associated with allergic reaction in vivo will be detected.

[0006] When the detection system uses antibodies (monoclonal or polyclonal antibody), the specificity or affinity of the antibodies are dependent on the type of IgE's used or the immune response of the animal used for immunization. In this case, when the antibody used for the assay show a different specificity, some specific IgE will be detected, but it will not detect all IgE. Especially, when the sandwich method of ELISA is utilized, two different type of antibodies must be used for detection. Therefore, the risk increases two fold, and different results will be obtained with different combinations of the antibodies.

[0007] In addition, there are inhibitory factors against specific antigen-antibody reactions in blood, which are assumed to affect the assay systems. Examples of these include: rheumatoid factor, NAF, bilirubin, hemoglobin, and chyle. In addition, it is considered that heparin, sodium oxalate, EDTA, and sodium citrate are included as inhibitory factors against antigen-antibody reactions.

[0008]
[Related techniques] The present inventors have already developed the expression of soluble FcεRI molecule using Baculovirus and insect cells and a large amount of soluble FcεRI molecule are now obtainable. In addition, our patent (patent 5-201991) describes that the construction of detection methods of IgE using the soluble FcεRI molecule is possible. However, a measurement of IgE in human blood has not been performed.

[0009]
[Problems attempted to be resolved by the invention] Therefore, the present invention supply the methods which selectively and specifically detect or measure IgE which possesses a possibility of triggering an allergic reaction using the soluble FcεRI molecule in vivo, especially in blood.

[0010]
[Method for solving the problems] As a result of examination of the aforementioned problem for a possible remedy, the present inventors established the methods for selectively and specifically detecting and quantifying IgE which has the capability of causing an allergic reaction, using a soluble α chain of FcεRI taking advantage of the fact it specifically and with high affinity binds to IgE molecules.

[0011] Therefore, the present invention supply the method for determining the presence or the quantity of IgE, based on the results of the measurement of the complex formed between IgE and a soluble α chain of FcεRI, which is obtained by incubating a soluble α chain of FcεRI with the diluted blood or blood component which is the sample of which the IgE is to be detected or measured.

[0012] One form of the method mentioned above is— anti-IgE antibody is secured onto a solid phase, and this solid phase is put into contact with the sample or the diluted sample, and if this sample contains IgE, this would be binded by the anti-IgE antibody which would be fastened to the solid phase. Next, this solid phase is put into contact with soluble α chain of FcεRI, leading to the formation of a bond between this soluble α chain of FcεRI and the solid phase. The presence or quantity of the soluble α chain of FcεRI determines the presence or quantity of the IgE in the sample.

[0013] Other form of the method mentioned above is— soluble α chain of Fc RI is secured onto a solid phase, and this solid phase is put into contact with the sample or the diluted sample, and if this sample contains IgE, this would form a bond with the soluble α chain of Fcε RI which would be fastened to the solid phase. Next, this solid phase is put into contact with anti-IgE antibody, leading to the formation of a bond between this anti-IgE antibody and the solid phase. The presence or quantity of the anti-IgE antibody determines the presence or quantity of the IgE in the sample. For the present invention, blood or blood components are those body fluid which include: whole blood, components isolated from the serum, plasma, liquid derived from bone marrow, lymph, urine and saliva.

[0014]
[Concrete explanation] Fcε RI is a glycoprotein which specifically binds IgE with a high affinity (Ka = 1010 M-1) and is expressed only on mast cells, basophils, and skin dendritic cells. As for molecules which bind to IgE, low affinity IgE receptor (Fc6RII, Ka=107 M^{-1}) which is expressed on B cell, T cell and macrophage. It is shown that FcγRII and FcγRIII which are low affinity IgG are also binded to aggregated IgE against aggregated IgG with the same affinity. However, only a receptor with high affinity for IgE is FcεRI.

[0015] FcεRI is a tetramer which consists of a chain, chain and 2 molecules of chain. Of these units, it is cleared the part associated with binding to IgE is α chain (Kinet JP 1990). α chain is membrane-protein going through the cell membrane, and has polysaccharide chains in vivo.

[0016] The extracellular domain of a chain has two immunoglobulin-like domain which leads to this polypeptide to be considered a member of the immunoglobulin superfamily. It is suggested that the second domain of these has essential parts for binding to IgE, however, since one domain can not sustain binding activity, it is thought that the binding site is decided by the three dimensional structure formed by the two domains.

[0017] The trans-membrane region of α chain FcεRI were deleted to make the recombinant FcεRI, and these were expressed using CHO cells. The chain was expressed as a soluble form and secreted outside the cells. These FcεRI had high affinity to IgE and this affinity was retained after the polysaccharide chain was removed. The present inventor were successful in producing soluble α chain of FcεRI using recombinant Baculovirurs and insect cells with high efficiency (patent 5- 201991). Also detection of IgE was achieved through the use of the this soluble α chain of FcεRI.

[0018] Additional expression system such as recombinant yeast fungi or animal cell (CHO) can be used for producing Fcε RI for detection of blood IgE. A soluble α chain of Fcε RI can be reliably obtained by using α chain of Fcε RI produced by the recombinant cells.

[0019] The characteristics of the present measurement method using specific bond between Fcε RI and IgE compared to that using antibodies are as follows: First, only IgE associated with allergy disease can be detected due to use for FcεRI. Second, binding of FcεRI and IgE involves high affinity, and this affinity is generally higher than that of antibody used in antigen-antibody reaction which will likely increase the sensitivity and decrease the detection time.

[0020] Thirdly, the following points may overlap with the first characteristic, but the complex of anti-IgE antibody produced in another animals such as mouse, rabbit and goat, and IgE do not occurred in vivo of human. However, binding of FcεRI and IgE is occurred in vivo of human. For example, when detect IgE serum as shown in the present invention, the present characterization could remove the effects of substances which inhibit antigen-antibody reaction in blood.

[0021] An IgE measuring method that uses the newly invented soluble α chain of FcεRI and IgE can be based on various immunoassays using specific antigen-antibody reactions. The methods of immunological measurements are described in detail in the Japanese text (name of text book). In this case, all the specific antibodies used in the methods of immunological measurement can be replaced with the α chain of FcεRI.

[0022] Sandwich system is considered as one of the methods of quantifying the total IgE in blood. For example, soluble α chain of FcεRI is bound to a carrier such as, microplates, beads, nitrocellulose membranes and nylon membranes used for enzyme-linked immunoassay(ELISA, EIA), or radioimmunoassy (RIA).

[0023] The binding methods should be selected on the basis of efficiency. Samples (serum) are put in contact with these carriers. For this procedure, sera etc can be incubated without pretreatment, but generally, the sample is diluted using a suitable dilution solution. This is done to prevent the absorption of non-functional IgE to the carrier, and allow the functional IgE of the sera that are capable of binding the soluble α chain of FcεRI bind it.

[0024] Next, non-binding parts are washed with the methods commonly used, and then anti-IgE antibody labeled with enzymes (for example, horseradish peroxides, β-D-galactosidase, alkalyl phosphatase), biotin, radioisotope etc are incubated to allow the binding of it to the IgE which is bound to α chain of FcεRI. After non-binding parts are washed with the methods commonly used, finally the functional IgE which is bound to a chain of FcεRI is detectable by detection of the labeled enzymes etc.

[0025] Conversely, antibodies specifically binding to IgE are bound to the carrier such as microplates, beads, nitrocellulose membranes, nylon membranes using the methods commonly used in enzyme-immunoassay (ELISA, EIA),and radio immunoassay (RIA). The binding methods should be selected on the basis of efficiency. Samples (serum) are incubated in these carrier. For this procedure, sera etc can be incubated without pretreatment, but generally, the sample is diluted using a suitable dilution solution. This is done to prevent the binding of substances that may cause non- specific reactions and also to allow all of the IgE contained in the sample to bind.

[0026] Next, non-binding parts are washed with the methods commonly used, and α chain of Fcε RI labeled with enzymes (for example, horseradish peroxides, β-D-galactosidase, alkalyl phosphatase), biotin, radioisotope etc are incubated to allow the binding of it to the IgE which is bound to the antibody. After non-binding parts are washed with the methods commonly used, finally the functional IgE which is bound to α chain of Fcε RI is detectable by detection of the labeled enzymes etc.

[0027] For either method, it is important for the antibodies used do not inhibit the complex formation between soluble a chain of FcεRI and IgE. Namely, because soluble a chain of FcεRI is known to bind to CH domain of IgE, choosing the antibodies that do not interfere with this is important. Also, these antibodies should be able to bind to the IgE which is already bounding to α chain of FcεRI, and is necessary to be able to detect all IgE and not only specific ones.

[0028] For the methods which detect total IgE in blood, competition radioimmunoassay (RIA) are considered. The characteristic of this method is that only soluble α chain of

Fcε RI will be bound to IgE, and secondary antibody will not be used. Further detailed explanation are described in our patent (5-218920). Another methods are explained in the textbook in detailed.

[0029] By use of the soluble α chain of FcεRI in this invention, total IgE in blood as well as specific IgE against individual antigens causing allergy can be measured. Antigens can be obtained from nature, or clone the genes to obtain recombinant antigens. The further detailed methods are explained in our patent (5-218920).

[0030] Further detailed methods are described the textbook (Japanese text book, p3-p60). The present invention enabled the measurement of total IgE associated with allergy and IgE against individual antigens. The usual methods are considered to entirely correlate with the present methods through the above explanations. However, when individual samples are examined, examples of discrepancy between measurement values is seen. These incidences indicate that this invented method specifically measures IgE and selectively detect IgE associated with allergy. By the use of this measurement method, it is considered that IgE closely associate with disease state and symptom can be detected. Furthermore, by measuring IgE against individual antigens, causative agents inducing allergy can be specified and lead to a useful course of therapy. Significant discrepancy detected in the present method compared to the usual methods is that the usual methods detect IgE not associated with allergy, or conversely did not detect the specified IgE in some cases. The measurement method of IgE using soluble α chain of FcεRI supply us useful information for diagnoses and therapies compared to usual methods.

[0031]
[Efficiency of the invention] The present invention enable us to selectively detect only IgE associated with allergic diseases. Also it can avoid the effects of the substances which inhibit antigen-antibody reaction in serum to a high degree by the use of soluble α chain of FcεRI, and have become able to construct a detection system similar to that of in vivo. This incidences are useful for diagnosis of variety of diseases such as, allergy diseases, parasite infection, severe hepatic debilitation, eczema and IgE myeloma.

[0032] Furthermore, the IgE detection system in the present invention is useful for evaluation of the efficacy of allergy therapies and the elucidation of Pathogenesis.

[0033] Further detailed usage examples are shown below. However, the present invention is not restricted to the following examples at all.

[0034] Example 1. Measurement of IgE in blood by coating anti-IgE antibody to a carrier (Anti-IgE antibody coating method)
(1)Anti-human IgE antibody coating to carrier
Anti-human IgE mouse monoclonal antibody (sigma) was dissolved to the concentration of 1.25μg/ml with 0.05M PBS (pH8.0) containing 0.25M NaCl. 100μl of the diluted

antibody were aliquoted to each well of the microplates (Nunc) and was incubated O/N at 4 C.

[0035] (2) Blocking of the microplates
Each well was washed twice with 330 µl of washing solution {0.1 M PBS (pH7.4) containing 2.7mM KCl, 137mM NaCl, 0.05%Tween20), and then blocked(stored) with 300 µl of blocking solution {phosphate buffer solution (0.1M phosphate buffer solution (pH 7.4) containing 2.7mM KC1, 137mM NaCl) containing 0.5%(w/v) casein sodium} for 2h at RT.

[0036] (3) Measurement of IgE

After each well was washed three times with 330 µ1 of the washing solution, 100 µl of dilution solution for samples (0.1 M phosphate buffer solution (pH 7.0) containing 0.5M NaCl, 1%BSA, 0.05%Tween 20) was added. 10 µl of Sample sera was added to each well and then were incubated for 2h at RT. Serum derived standard IgE (2000U/ml) was measured as standard IgE. The standard IgE was two-fold serially diluted with the sample dilution solution, and 10 µl of it were added to each well and were incubated for 2h at RT. The samples exceeding the measurement range were re-measured after being diluted to 10 fold or 100 fold with the dilution solution.

[0037] After each well was washed three times with 330 µl of the washing solution, biotin labeled soluble α chain of FcεRI (0.1 mg/ml) was dilute to 1: 1000 with the dilution solution for labeling, and 100 µl of it was added to each well and incubated for 1 h at RT. After the wells were washed with 330 µl of the washing buffer, 100 µl of 5000 fold-diluted horseradish-peroxidase labeled avidin (1 mg/ml) diluted with the dilution solution for labeling were added and were incubated for l h at RT.

[0038] Next, after the wells were washed four times with 330 µl of the washing buffer, OPD tablets (Wako) were dissolved in 50 ml of the dissolution solution (0.1 M phosphate buffer (pH5.0), 0.009% H_2O_2) and 100 µl of that were added to each well and were incubated for 30 min in a dark room at RT. Reaction was stopped with 2N H_2SO_4. The absorbance of each well was measured at 492 nm by a microtiter-plate reader. Figure 2 showed the curb of two-fold serially diluted serum-derived standard IgE (2000 U/ml). A nice dilution line was obtained. When the quantity of IgE in a sample is actually measured, the quantity of IgE in sample was calculated using this standard curb.

[0039] (4) Reconfirming test
Reconfirming test was carried out under the methods described above. Serum containing 2030 IU/ml was two-fold serially diluted with the sample dilution solution, the test was carried out under 2030, 1015, 508, 128 and 0 (only the solution) IU/ml, and three unknown samples (n=8). As shown in Table 1, a nice reconfirming result was obtained

with CV value 6.37% at maximum. This incidence indicate that the detection system using soluble α chain of FcεRI is a very stable system when measure IgE in blood.

[Table 1]

[0041] Example 2. Measurement of IgE in blood by carrier coating the soluble a chain of Fcε RI (Fcε RI a chain coating methods)

(1) Coating of soluble α chain FcεRI to carrier

Soluble α chain FcεRI were dissolved to make the concentration of 2 μg/ml with 0.5M sodium phosphate buffer (pH9.6). 100μl of this was aliquoted to each well of the microplates (Nunc) and was incubated O/N at 4 C.

[0042] (2) Blocking of microplates

After each well was washed twice with the washing buffer (0.1 M phosphate buffer containing 2.7mM KCl, 137mM NaCl, 0.05%Tween 20), 300 μl of the blocking solution (0.1 M sodium phosphate buffer composed of phosphate buffer (2.7mM KCl and 137mM NaCl) containing 0.5%w/v sodium casein was added to each well and incubated for 2h at RT.

[0043] (3) Measurement of IgE

After each well was washed with 330 μl of the washing buffer, 100 μl of the sample dilution solution (0.1 M phosphate buffer (pH7.0) containing 0.5NaCl, 1%BSA were added. 10 μl of samples were added to the wells and were incubated for 2h at RT. As standard IgE, IgE derived from serum (2000 U/ml) were measured. The standard IgE was two-fold serially diluted with the sample dilution solution, and 10 μl of it were added to each well and were incubated for 2h at RT. The samples exceeding the measurement range were re-measured after being diluted to 10 fold or 100 fold with the dilution solution.

[0044] After each well was washed three times with 330 μl of the washing solution, horseradish peroxidase-labeled goat anti-human IgE polyclonal antigen was diluted 2000 fold with the standard dilution solution (0.1M phosphate buffer (pH7.4) containing 0.15 M NaCl, 1 % BSA}. 100 μl of this was added to each well and were incubated for 1 h at RT. Next, after each well was washed four times with 330 μl of the washing solution, OPD tablets were dissolved in 50 ml of the dissolution solution (0.01%H_2O_2, 0.1M sodium phosphate buffer), and 100 μl of this was added to each well and were incubated for 20 min at RT in at dark room.

[0045] After that, reactions were stopped using 2N H_2SO_4. The absorbency of each well was measured at 492nm using Microplate reader. Figure 2 showed the lineality of two-fold serially diluted serum-derived standard IgE (1000 U/ml). A nice dilution line was obtained. When the quantity of IgE in a sample is actually measured, the quantity of IgE in sample was calculated using this standard curb.

[0046] Example 3. Measurement of IgE in sera of allergy patient with usual-method, anti-IgE antibody coating method (Example 1) and soluble α chain of FceRI coating method (Example 2). Serum IgE of allergy patient or normal human was measured using: usual method (Eldia method), anti-IgE antibody coating method (Example 1: FcR method) and, soluble α chain of FcεRI coating method (Example 2: FcR coating method).

[0047] Usual method used was the Eldia method (Kokusai shiyaku), and the measurement was done by automatic measuring machine (Eldia auto) according to the instruction manual. FcR method and FcR coating method were measured by methods described in Example 1 and Example 2, respectively. The standard IgE used was IgE that was supplied with Eldia IgE, and the standard lines were drawn with measurements in each method. Over 90 samples including allergy patients and normal human sera were measured, and the samples exceeding the measurement range were re-measured after being diluted with the dilution solution. Table 2 and 3 showed measurement values in three methods.

[0048]
[Table 2]

[0049]
[Table 3]

[0050] Figure 3 and 4 showed correlation of measurement values between Eldia method and FcR method, and correlation of measurement values between Eldia method and FcR coating method, respectively. In addition, Figure 5 showed the correlation of the measurement values between FcR method and FcR coating method. The correlation of each measurement value is relatively significant and the correlation coefficient was 0.949, 0.981 and 0.983 respectively. However, the number of units in few samples deviated from each measurement method.

[0051] Especially, the FcR method and FcR coating method did not have many discrepancies, but discrepancies were seen in Eldia method and methods using the a chain of Fcε RI (Figure 3 and 4). This was due to the fact, only active IgE that is capable of binding to Fcε RI was detected by the measurement method using soluble a chain of FcεRI. In addition, it is considered that the effects of substances which inhibit antigen-antibody reaction in blood could be negated.

[0052] Example 4. Measurement of blood IgE using RIA method, Eldia method, FcR method and FcR coating method. Results from measurement values obtained from RIA method which is one of the IgE detection systems, was compared to that of Eldia method, FcR method and FcR coating method. RIA method was measured by using Pharmacia IgE RIA(RIST method: Pharmacia Biosystems). One hundred thirty samples which had been measured by RIST method were measured by Eldia method, FcR method and FcR coating method. The results are shown in Table 4 and 5.

[0053]
[Table 4]

[0054]
[Table 5]

[0055] Similar to Example 3, measurement values of IgE in four methods correlated. However, some samples (No. 85 and 112) had discrepancies. It was considered that these were strongly indicated usefulness of the system using soluble α chain of FcεRI.

[0056] Reference 1. Biotin labeling of soluble α chain of FcεRI
The biotinylation of soluble α chain of FcεRI purified from Baculovirus-expression vector was carried out as follows. Purified soluble α chain of Fc RI was dissolved to make 0.2mg/ml with 0.2M sodium carbohydrate and 0.2 M NaCl. To 2.9 ml of this solution, 30 μl of NHS-LC-Biotin (Pierce) was added and was incubated with gentle stirring for 2h at RT. Furthermore, 0.2M glycine solution was added and was incubated with gentle stirring for 1 h at RT. Biotin labeled soluble α chain of FcεRI were obtained by dialyzing this solution with 0.2M NaCl and 20 mM Tris-HCl buffer (pH 7.6) and eliminating the reaction reagents.

[0057] Reference 2. Preparation of plasmid that has gene fragment encoding human soluble a chain of FcεRI inserted. Gene fragments encoding human soluble a chain of Fc RI were obtained by: synthesizing a primer from plasmid BS-SK(+) her a which contains and inserted gene fragment encoding human soluble a chain of FcεRI using DNA synthesizer (8700DNA synthesizer, MilliGen/Biotech), and through the use of PCR. The primer made were :

NTV primer; 5' -AAAAACTCGAGATGGCTCCTGCCATGGAATCCCCTACT(sequenceNo.1)
PAS primer; 5'- AAAAAGGATCCTTAAGCTTTTTATTACAGTAATGTTGA(sequence No.2)

[0058] PCR was carried out under the following condition. 100 μl of the reaction solution (1ng plasmid BS-SK (+)heraDNA, 1 μM NTV primer, 1 μM PAS primer, 10 mM Tris-HCl (pH 8.3) 50mM KCl, 1.5mM $MgCl_2$, 0.001% gelatin, 200 μM dATP, 200 μM dCTP, 200 μM dGTP, 200 μM dTTP, 2.5 U amplitaq DNA polymerase (TAKARA)) were subjected to 30 cycles of 1 min at 94 C, 2 min at 50 C, 3 min at 72 C using DNA Thermal Cycler (PerkinElmer Cetus).

[0059] 20 μl of the reaction solution were loaded in 0.8% agarose gel. The DNA fragment of 0.6 kb purified from the gel using GeneClean (Bio 101) was recovered in 16μl of TE (10mM, TrIS-HCl, 1mM EDTA). 2μl of 10× reaction buffer solution (10 mM Tris-HCl (pH 7.5), 1.5M NaCl, 70mM MgCl) were added and then 1μl of Bam HI and XhoI, respectively, were also added and was incubated 1h at 37 C.

[0060] After the enzymes in the reaction solution were heat-inactivated for 10 min at 65 C, 5 μl of this was ligated to 10 ng of the cloning Vector p3TU19X (patent 3-106600) cut using Bam HI and XhoI. The reaction was carried out for 1h at 16 C using DNA ligation kit (30 μl of A solution and 6 μl B solution).

[0061] 10 μl of the DNA solution described above were cultured in L-amp media. *E. coli* XL-1Blue was transformed according to Hanahan method and were plated on L-amp plates and amp-resistance and β-galctocidase deficient colonies were selected by choosing white colonies.

[0062] Recombinant phage was prepared by infecting the selected colonies grown with L-amp media with Helper phage VCS13. Single strand DNA was prepared from the recombinant phage, and 0.8 μg of the DNA were reacted with DNA sequence system/Taq polymerase according to the methods recommended by the company and the reaction solution was analyzed using DNA sequencer Model 370A (version 1.30 ABI) and the sequence was determined.

[0063] 1 μg of the recombinant plasmid DNA prepared by Alkaline lysis method was incubated with M13RPI primer according to the methods recommended by the company and the reaction products were analyzed (version 1.30 ABI) and the sequences were determined.

[0064] The sequences of recombinant plasmid pNTV detemined by both method described which had a part of the structural gene, was not different from the human α chain of FcεRI, and XhoI site was imported from the NTV primer before the ATG codon. In addition, the sequences of the plasmid had stop codon TAA and BamHI site after the region coding for the 172th amino acid which was recognized when the coding region for the 25th amino acid valine was considered to be the 1st amino acid.

[0065] Reference 3. Isolation of human α chain of FcεRI gene fragment
RNA was extracted from KU812 cell (4×10^7) using total RNA separator kit (Clonetech) according to the protocol recommended by the company. Poly A RNA was purified from the extracted RNA using mRNA separator kit (Clonetech) according to the protocol recommended by the company. 40 μg of the polyA mRNA were obtained. Double stranded cDNA was synthesized using 5 μg the poly A mRNA using Time saver DNA sysnthesis kit (Pharmacia) according to the protocol recommended by the company.

[0066] 100 μl of the reaction solution {1 μM NTV primer, 1 μM PAS primer, 10 mM Tris-HCl (pH 8.3) 50mM KCl, 1.5mM $MgCl_2$, 0.001% gelatin, 200 μM dATP, 200 μM dCTP, 200 μM dGTP, 200 μM dTTP, 2.5 U amplitaq DNA polymerase (TAKARA)} was subjected to 30 cycles of 1 min at 94 C, 2 min at 50 C, 3 min at 72 C by DNA Thermal Cycler using 10 μl of the cDNA obtained and PAS primer and NTV primer as described above.

[0067] 20 μl of the reaction solution were loaded in 0.8% agarose gel. The DNA fragment of 0.6 kb purified from the gel using GeneClean (Bio 101) were recovered in 16 μl of TE (10mM, Tris-HCl, 1mM EDTA). 2μl of ×10 reaction buffer solution (10 mM Tris-HCl (pH 7.5), 1.5M NaCl, 70mM MgCl) were added and then 1μl of Bam HI and XhoI, respectively, and was incubated 1h at 37 C.

[0068] After the enzymes in the reaction solution were heat-inactivated for 10 min at 65 C, 5 μl of this were ligated using 10 ng of the cloning Vector pBlurscriptIIKS(+) (cut with Bam HI and XhoI) for 1h at 16 C with DNA ligation kit (30 μl of A solution and 6 μl B solution). 10 μl of the DNA solution described above were cultured in L-amp media. *E. coli* XL-1Blue was transformed according to Hanahan method and were plated on L-amp plates and amp-resistance and β-galctocidase deficient colonies were selected by choosing white colonies.

[0069] 1.5 μl of the plasmid DNA prepared from the colonies were incubated using Dye primer cycling sequence kit (ABI) and M13RP1 or -21M13 primer according to the methods recommended by the company and the reaction products were analyzed using DNA sequencer Model 370A (version 1.30 ABI) and the sequences of the insert fragment were determined.

[0070] The inserted fragments had the same sequence as reported previously, and XhoI site was imported from the NTV primer before the ATG codon. In addition, the sequences of the plasmid had stop codon TAA and BamHI site after the region coding for the 172th amino acid which was recognized when the coding region for the 25th amino acid valine was considered to be the 1st amino acid. It is clear that the plasmid obtained is replaceable with pNTV in Reference 2.

[0071] Reference 4. Production of the expression pladmid of human soluble α chain of FcεRI

1 μg of plasmid pNTV2 (containing human soluble α chain of FcεRI gene) was incubated in the reaction solution {10mM Tris-HCl(pH7.5), 100mM NaCl, 7mM $MgCl_2$, 12 U XhoI, 10UBamHI) for 1h at 37 C.

[0072] After reaction, DNA was extracted with phenol/chloroform and was recovered by ethanol precipitation. The DNA fragments were incubated with DNA blunting end kit (TAKARA) according to the methods recommended by the company. The reaction solution was loaded in 0.8% agarose gel. The DNA fragment of 0.6 kb purified from the gel using GeneClean (Bio 101) was recovered in 10μl of TE.

[0073] Separately, 1 μg of pAcYM1 which is Baculovirus expression vector (Matsuura, y et al., L. Gen. Viro. 68. P1233-1255) was incubated in 10 μl of the reaction solution {10mM Tris-HCl(pH7.5), 100mM NaCl, 7mM $MgCl_2$, 10U BamHI} for 1h at 37 C. The reaction solution was loaded in 0.8% agarose gel. The DNA fragment of 10 kb purified from the gel using GeneClean (Bio 101) was recovered in 10μl of TE.

[0074] 2 μl of the solution containing the fragments mentioned above and 3 μl of the solution containing fragments recovered from pNTV were separately mixed with DNA ligation kit (25 μl of A solution and 5 μl B solution) for 1h at 16 C, and both DNA were ligated. 10 μl each of the DNA solution described above were cultured in L-amp media. *E. coli* XL-1Blue was transformed according to Hanahan method and were plated on L-amp plates and amp-resistance and β-galctocidase deficient colonies were selected by choosing white colonies. Restriction enzymes sites of the plasmid DNA of the selected colonies were observed, and pAcYM-NTV which is Baculovirus expression vector, was obtained by the selection of transformant having ligated individual fragment.

[0075] Reference 5. Isolation of expression recombinant virus with the human soluble α chain of FcεRI

10 μg pAcYM-NTV, which is Baculovirus expression vector and 1μg of natural type Baculovirus, were inoculated into Sf-9 cell which is *Spodoptera frugiperda* culture cell. The methods of infection was done according Matsuura et al.,(Japanese textbook). After inoculation, plaques not forming polyhedrin were selected by plaqueassay. Recombinant virus were finally isolated by performing plaqueassay three times.

[0076] The isolated viruses were inoculated into Sf-9 with MOI(multiplicity of infection)=1 and were incubated in Grace media (Gibco) containing 10%FCS for 72h. Ac-NTV which is expression recombinant virus of the human soluble α chain of FcεRI, was isolated by analyzing part of the supernatant of the media using the Western blot method. The isolated virus were inoculate into Sf-9 cell with MOI(multiplicity of infection)=1 and were incubated in Grace media (Gibco) containing 10%FCS for 72h, and approximately 1×10^8 pfu/ml recombinant virus were obtained by recovery of the supernatant of the media.

[0077] Reference 6. Expression and purification of expressed products

The recombinant virus were inoculated into Sf-9 (4×10^8) cell with MOI(multiplicity of infection)=5, and were incubated in 400 ml of Grace media (Gibco) containing 10%FCS for 72h, and the supernatant of the media was recovered. Saturated $(NH_4)_2SO_4$ was added to the supernatant to be 60% saturated, and was incubated for 2h at 4 C. The sediment was recovered after centrifugation for 30min at 10000rpm.

[0078] After the recovered sediment was dissolved in TBS {20mM Tris-HCl,(pH7.5), 150mM NaCl}, it was dialyzed O/N at 4 C. After dialysis, the solution was recovered and was separated in TBS using Sephacryl 200 (2.6 cm × 60 cm) equalized by TBS. The fraction corresponding to sizes ranging from 30 kDa to 40 kDa were collected.

[0079] Preparation of anti- FcεRI monoclonal antibody column was done by the following methods. BrCN-activated sepharose 4B (Pharmacia) was soaked in 1mM HCl for 15 min at RT. After the distended gel on a glass filter were washed with 1mM HCl

and soaked repeatedly, it was washed with the coupling buffer solution{0.5 M NaCl, 0.1M $NaHCO_3$(pH8.3)}.

[0080] Immediately, 2 ml of this gel was added to 2 ml of purified FcεRI monoclonal antibody dissolved in the coupling buffer solution and was incubated for 2h at RT with gentle stirring. After the supernatant was removed, the gel was suspended in 0.2 M glycin-HCl (pH8.0) and was incubated for 2h at RT with gentle stirring. Next, this gel was washed with 0.1 M acetate buffer solution (pH4.0) containing 0.5 M NaCl and the coupling solution repeatedly and anti-FcεRI monoclonal antibody column was obtained.

[0081] The purification of the fraction obtained from the gel filtration using the anti-FcεRI monoclonal antibody column was carried out under the following condition. The fractions containing of the FcεRI obtained from the gel filtration were added to the anti-FcεRI monoclonal antibody column equalized with 20mM Tris-HCl (pH7.6) containing 0.5M NaCl and were washed with the buffer solution which was used for equilibration.

[0082] Next , elution was carried out using 20 mM Tris-HCl (pH7.6) containing 3M sodium thiocyanate and 0.5M NaCl and eluted fractions were immediately dialyzed with 20 mM Tris-HCl (pH7.6) containing 0.1M NaCl, and the purified standard product of soluble soluble α chain of FcεRI was obtained.

[0083] Reference 7. Purity check of the purified standard product
The purity check of the purified standard product was carried out under the following condition. The purified standard product was concentrated and then was resuspended in sample buffer (62.5 mM Tris-HCl (pH6.8) containing 2% SDS, 5% 2-ME, 7% glycerol, 0.005% and bromophenol blue) and was boiled for 5 min at 100 C.

[0084] The boiled sample was loaded in 10-20% gradient SDS-PAGE gel. The gel was stained with silver stain kit (Daiichi Kagaku) and detection of the protein was carried out. As results, a single band located around the region of 30 kDa ~35 kDa was detected.

[0085]
[Table of the sequence]
Sequence number: 1
Sequence length: 38
Sequence type: nucleic acid
Strandedness: single
Topology: linear
Sequence type: synthetic DNA

Sequence
AAAAACTCGA GATGGCTCCT GCCATGGAAT CCCCTACT 38

[0086]

Sequence number: 2
Sequence length: 37
Sequence type: nucleic acid
Strandedness: single
Topology: linear
Sequence type: synthetic DNA

Sequence
AAAAAGGATC CTTAAGCTTT TATTACAGTA ATGTTGA 37

[Simple explanation of figure]
[Figure 1] Figure 1 showed the graph depicting the result of absorbance of two-fold serially diluted serum-derived standard IgE (2000 U/ML) measured at 492 nm using the method of Example 1 (anti-IgE antibody coating method) of the present invention.

[Figure 2] Figure 2 showed the graph depicting the result of absorbance of two-fold serially diluted serum-derived standard IgE (1000 U/ML) measured at 492 nm by the method of Example 2 (FcεRI α chain coating method) of the present invention.

[Figure 3] Figure 3 showed the graph depicting the correlation between measurement values of the quantity of human blood IgE by Eldia method which is a usual method, and that by FcR method of the present invention.

[Figure 4] Figure 4 showed the graph depicting the correlation between measurement values of the quantity of human blood IgE by Eldia method which is a usual method, and that by FcR coating method of the present invention.

[Figure 5] Figure 5 showed the graph depicting the correlation between measurement values of the quantity of human blood IgE by FcR method of the present invention, and that by FcR coating method of the present invention.

From the first page

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